Name: Protyusha Dey
Department- Biotechnology
Registration number- VB-1679 OF 2012-13

Synopsis Title

Cloning and partial characterization of a gene encoding endo-1, 4-β-xylanase from *Termitomyces clypeatus*

Introduction

Fungi play a vital role in carbohydrate metabolism by producing an extensive set of carbohydrate degrading enzymes which have important industrial uses. Most of these enzymes in fungi are constitutive, but sometimes they are found to be adaptive, being synthesized only in the presence of substrates. Among fungal classes, Basidiomycetes are known to be efficient degraders of hemicellulose. Many of these species grows on dead wood or litter. *Termitomyces clypeatus* is one such basidiomycetes fungus which grows in association with termites. The fungus helps the termites to degrade the plant-derived materials (eg., wood, dry grass, and leaf litter) on which they live. *T. clypeatus* is known as a powerful producer of different carbohydrate degrading enzymes (Mukherjee and Sengupta , 1985, Khowala & Sengupta , 1992) like amylase, xylanase, cellulose etc. These industrially important carbohydrate splitting enzymes have been purified and characterized biochemically from the fungus.

Xylan, the major constituent of the hemicellulose, is also the second most abundant natural polysaccharide that accounts for approximately 1/3 of the renewable organic carbon on earth. It is primarily present in the secondary cell wall together with cellulose and lignin (Motta et al.). Xylan is made up of xylose, a pentose sugar. The primary chain of xylan is composed of beta xylopyranose residues joined with beta 1,4- linkages. The xylopyranosyl residues are often found to be substituted with arabinose, glucuronic acid, arabinoglucuronic acid making xylan heterogeneous and structurally diverse.

The enzymatic hydrolysis of xylan and the resulting xylanolytic products have got a huge number of industrial uses which includes their application in biofuel production, paper and pulp industries, food and feed industries, pharmaceutical industries etc. As the structure of xylan is found to be diverse, a variety of enzymes are needed to hydrolyze xylan completely and the cluster of these enzymes is named as xylanolytic enzymes. This cluster includes endo-1,4-β-D-xylanase (EC3.2.1.8), β-xylosidase, α-glucouronidase, α-arabinofuranosidase, acetyl xylan transferase etc. The most important one is found to be the endo-1,4-β-D-xylanase (EC3.2.1.8) as they breakdown the backbone of xylan. These enzymes are the members of Glycoside Hydrolase (GH) family.

Roy 21/07/2018
सहायक अध्यापक
जैवप्रयुक्ति विद्या विभाग
विश्वभारती, शान्तिनिकेतन
Associate Professor
Department of Biotechnology
Visva-Bharati, Santiniketan

forwarded to library for uploading
31/7/18
अध्यापक एवं विभागीय प्रधान
जैवप्रयुक्ति विद्या विभाग
विश्वभारती, शान्तिनिकेतन
Professor & Head
Department of Biotechnology
Visva-Bharati, Santiniketan

Objectives

To meet the industrial needs researchers are interested in isolating and producing xylanase by finding new and more potent sources or by creating mutant strains (Gupta et al., 2000, He et al., 2009, Luo et al., 2010) of the enzyme. Previous works suggest that the fungus *T. clypeatus* secretes endoxylanase in the presence of xylan in the growth medium (Mukherjee et al., 1985). There is no report regarding the cloning and characterization of the gene. The objective of the proposed dissertation work is to clone the endo-1,4-β-xylanase gene and characterize it.

Proposed plan of the work

1. To design gene specific primers for amplifying the coding sequence of endo-1,4-β-xylanase gene of *Termitomyces clypeatus.*

2.Use the gene specific primers to obtain full length cDNA of the xylanase (from the total mRNA) gene by using RT-PCR.

3.To clone the cDNA in suitable *E. coli* vector followed by expression of the cloned gene.

4.To characterize the cloned gene and its expressed product biochemically and genetically.

Reference

Mukherjee M., Sengupta S., An Inducible Xylanase of the Mushroom *Termitomyces clypeatus* Differing from the Xylanase/Amylase Produced in Dextrin Medium Journal of General Microbiology (1985), 131, 1881-1885. Printed in Great Britain

He J, Yu B., Zhang K., Ding X., Chen D Expression of endo-1, 4-beta-xylanase from *Trichoderma reesei* in *Pichia pastoris* and functional characterization of the produced enzyme BMC Biotechnology 2009, 9:56 http://www.biomedcentral.com/1472-6750/9/56

Gupta N., Reddy V.S., Maiti S., Ghosh A. Cloning, Expression, and Sequence Analysis of the Gene Encoding the Alkali-Stable, Thermostable Endoxylanase from Alkalophilic, Mesophilic *Bacillus sp.*Strain NG-27 Applied And Environmental Microbiology,0099-2240/00/$04.0010 June 2000, p. 2631– 2635 Vol. 66, No. 6

Luo H., Yang J., Li J., Shi P., Huang H., Bi Y., Fan Y., Yao B. Molecular cloning and characterization of the novel acidic xylanase XYLD from *Bispora sp.* MEY-1 that is homologous to family 30 glycosyl hydrolases Appl Microbiol Biotechnol (2010) 86:1829–1839 DOI 10.1007/s00253-009-2410-0

Motta F.L., Andrade C. C. P. and SantanaA M. H. A. Review of Xylanase Production by the Fermentation of Xylan: Classification, Characterization and Applicationshttp://dx.doi.org/10.5772/53544.